IN THE DRAWINGS:

Please replace drawing sheets 1, 2, and 5-27 with the corresponding substitute drawing sheets.

REMARKS

Status of the application and Amendments

Claims 1-30, and 59-65 are pending in the application. With entry of this amendment, claims 1-5, 11-15, 17-19, 27-30, 59, 61, and 65 have been amended, the drawings have been renumbered, and the specification has been amended in accordance with the new drawing numbering. Also, substitute drawing sheets have been submitted herewith to reflect the new drawing numbering.

The amendments are made for improved clarity or for purposes of expediting prosecution, and should not be viewed as an acquiescence in any ground of rejection. No new matter has been introduced by the amendments.

With reference to the paragraph numbering in the Office Action, the following remarks address the other issues raised in the Office Action.

2. Restriction Requirement

Applicants affirm the provisional election with traverse of Group I, claims 1-26, and 59-64 that was made during a telephone conversation between Applicants' Representative, Mr. Bill Smith, and the Examiner on May 15, 2000. Applicants respectfully traverse the restriction requirement for the reasons stated below.

According to the MPEP, there are two criteria for a proper Restriction Requirement: (1) the inventions must be independent or distinct as claimed, and (2) there must be a serious burden on the Examiner if restriction is not required (MPEP § 803).

Applicants submit that the restriction requirement is improper because the restricted claims are closely related that they should remain in the same application to preserve unity of invention. Group I claims are directed to methods and apparatuses for amplifying

nucleic acids. Group II claims are directed to nucleic acid products that can be produced by methods recited in Group I claims and a kit comprising such nucleic acids.

As to burden to the Examiner, no prima facie case for serious burden was established in the Office Action. Because the claims are closely related, it is natural and reasonable and desirable to search prior art for these claims together. By no means would such search present serious burden on the Examiner.

In view of the foregoing, Applicants request reconsideration and withdrawal of the restriction requirement. Applicants expressly reserve the right to file subsequent applications claiming the non-elected subject matter and do not waive any of their rights or abandon any non-elected subject matter.

3. Priority

Applicants acknowledge with appreciation the Examiner's suggestion that all priority documents be referenced in the specification. The specification has been so amended.

4. Drawings

It is acknowledged that the drawings are being objected to. Applicants will correct any defects in the drawings upon a notice of allowable subject matter.

5. Compliance with Sequences Rules

Applicants will comply with the Sequence Rules by submitting a Sequence Listing under a separate cover.

6. Drawing numbering

The Office Action raised issues with informalities in description and numbering of the figures in the subject specification. In response, the Figures have been renumbered as follows:

Old Number	New Number
1-1	1A
1-2	1B
2	2
3	3
4a	4
4b	5
5-1	6A
5-2	6B
6a	7
6b	8
7-1	9A
7-2	9B
8-1	10A
8-2	10B
9	11A and 11B
10-1	12A
10-2	12B
10-3	12C
11-1	13A
11-2	13B
12-1	14A
12-2	14B
13	15
14	16
15	17
16	18
17	19
18	20
19	21

Substitute sheets of drawings have been submitted herewith to show the change of numbering. No other changes have been made to the drawings. The brief descriptions of drawings at pages 18-19 have been amended in accordance with the new numbering. References to figures throughout the specification have also been amended. In addition, Applicants note that at page 46, line 14, figure 16c should be figure 10c (now Figure 12B, (c)). Also, as noted by the Examiner, at page 68, line 5, figure 21b should be figure 18b (now Figure 20, (b)). These errors have also been corrected by amendments to the specification noted above.

No new matter has been introduced by the substitute sheets of drawings and the amendments to the specification. Applicants believe these amendments have corrected the informalities in the subject specification as noted by the Examiner.

7-9. Claim objections

Applicants have amended claims 1 and 2 to delete the period after the label of each step.

Claim 17 has been amended to insert an "or" as suggested by the Examiner.

Claims 62-64 are objected to as being dependent from a non-elected claim. Claim 61 has been amended to incorporate language of the non-elected claim 27 and is not dependent from claim 1. As such, claims 62-64, which depend from claim 61, no longer depend from a non-elected claim.

9-16. Claim rejections – 35 U.S.C. § 112

9-11. Claims 3-6 are rejected as allegedly vague and indefinite in the recitation of "wherein said single-stranded target nucleic acid is produced by providing a given nucleic acid sequence to be amplified". The Examiner says that it is unclear what the noted words mean in the claims, and that the rejection can be overcome by clarifying its meaning.

In response, Applicants have amended claims 3 and 4 by reciting "wherein said single-stranded target nucleic acid comprises a given nucleic acid sequence to be amplified to which has been added a first nucleic acid sequence and a second nucleic acid sequence....". Such amendment makes clear that "a given nucleic acid sequence" refers to a sequence to be amplified rather than the immobilized primer or the amplified product. Such meaning is further evident from the exemplary embodiment as shown in Figure 1-1, (b) (now Figure 1A, (b)). In this embodiment, the "given nucleic acid sequence to be amplified" is the central part of the gray bar (i.e., ...CCG...). To this "given nucleic acid sequence to be amplified", a first nucleic acid sequence (AAT) and a second nucleic acid sequence (ATT) has been added. The first nucleic acid sequence (AAT) hybridizes to one of the immobilized primers (TTA), and the second nucleic acid sequence (ATT) is complementary to a sequence (e.g., AAT) that hybridizes to one of the primers (e.g., TTA).

In light of the claim amendment and remarks, Applicants submit that claims 3-6 as amended are not vague or indefinite. Withdrawal of the instant rejection is respectfully requested.

12. Claim 5 has been amended to replace "first and second ends" with "3' and 5' ends", as suggested by the Examiner. Accordingly, the rejection has been overcome.

13. Claims 19-21 are rejected on the alleged grounds that the recitation of "part" in the claims is vague and indefinite. It was stated in the Office Action that it is not clear what "part" means in the rejected claims. The Examiner says that the rejection can be overcome by clarifying the meaning of "part" in claims 19 and 21.

Applicants submit that use of the word "part" is in no way indefinite and the broad language is justified. As highlighted by the embodiment of claim 20, the "nucleic acid molecule or a part thereof' may be released by cleavage with a restriction endonuclease or ribozyme. Thus, the part could be of any size – the size would only be dictated by the location of the restriction site. Withdrawal of the instant rejection is respectfully requested.

14. Claim 62 is rejected as allegedly being indefinite in the recitation of "so that another nucleic acid strand is located on the surface within a distance of the length of that strand". The Examiner says that the rejection can be overcome by clarifying the meaning of "that strand" in the noted phrase. In response, Applicants point out that, as recited in the claim, all strands are either identical or identical complements, and that they must all be the same length. Therefore, there is no lack of clarity: the meaning of "that strand" is the "identical nucleic acid strand" or "identical complementary strand". As such, Applicants submit that the instant rejection is overcome.

15. Claims 11 and 12 are rejected as allegedly being indefinite in the recitation of "substantially". To expedite prosecution, Applicants have deleted the objected wording from claim 12 and amended claim 11 by replacing "substantially" with "about". Applicants submit that there is no indefiniteness in the recitation of "about" as it is readily apparent that the different types of primers do not need to be in exactly identical concentrations in order for one to practice the claimed methods, and that acceptable variation in the concentrations of the different types of primers are well within the knowledge of the skilled artisans.

16. Claim 61 is rejected as lacking antecedent basis in the recitation of "claim 27" as claim 27 is a non-elected claim. Applicants have amended claim 61 to incorporate elements of claim 27, and the amended claim 61 no longer recites "claim 27".

17-18. Claim Rejections - 35 U.S.C. § 103(a)

Claims 1-26 and 59-64 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Hosoi et al. (EP-A-0665 293 A2) in view of Cheng et al. (Nucleic Acids Research, 24: 380-385, 1996) and Hahn et al. (Analytical Biochemistry, 229: 236-248, 1995). It was stated in the Office Action that the combined teachings of the cited art would have motivated one "to perform PCR on the solid support such as a capillary plate immobilized with a plurality of identical or different primers using single stranded nucleic acid templates and fluorescent-dNTPs, cleave PCR products by a specific restriction enzyme . . . ". As such, the Examiner is of the opinion that the presently claimed invention is obvious over the cited art. This rejection is respectfully traversed for the reasons stated below.

The present invention relates to nucleic acid amplification that uses extended, immobilized primers as templates for further rounds of primer extension on a surface (see, e.g., claim 1B-1D). This is illustrated in the figures of the present application, e.g., Figure 1A, where extended immobilized primers can be seen leaning over to form "bridge-like" structures. Thus, the extended, immobilized primers themselves hybridize with adjacent primers. By contrast, as discussed below, none of the cited references teaches or suggests, among others, this feature of the presently claimed methods.

The Hosoi et al. reference does not relate to amplification, but to a particular method of sequencing by primer extension, which includes a step of measuring the amount of growth of primers. The extended primers disclosed in Hosoi are simply detected without being themselves used as templates for further rounds of primer extension. There is nothing in Hosoi to suggest providing pairs of PCR primers in immobilized form located sufficiently close to each other (within the length of an extended primer) so that an extended immobilized primer can act as a template for further rounds of amplification in an immobilized system. On the contrary, Hosoi provides the different primers in separate wells. The wells are clearly too far

from each other for "bridges" to be formed between the different wells so as to allow extended immobilized primers to hybridize to further primers (see e.g. Figure 1).

In addition, Hosoi teaches against mixing primers at a given location (see e.g. page 3, lines 51 to 54) and it is therefore clear that the method of Hosoi could not be used for the present invention (where forward and reverse PCR primers would need to be located very close to one other on a surface, i.e., not at separate locations).

Further, in order to sequence a molecule according to Hosoi et al., all possible primers against a given region must be made and placed in precisely defined separate regions. Thus, the plates used in Hosoi et al. for sequencing are expensive and time-consuming to manufacture (see e.g. Figures 1 and 2, as well as the abstract and claim 1).

Cheng et al. discuss a method involving repeated cycles of nucleic acid amplification. However, Cheng et al. also teach away from the presently claimed methods which involve immobilization for amplification. Cheng et al. discuss use of silicon-glass chips for PCR amplification. It is important to note that the chips are not used for immobilization of primers on a surface, but for a completely different purpose. They are used to provide an environment in which there is a high SVR (surface to volume ratio) in order to allow for "more efficient thermal conduction and dissipation". This is clear, e.g., from the following passage taken from the "Results and Discussion" section at page 382 of Cheng et al.:

> "A feature of the chip is its high surface to volume ration [210 mm^2:12 µl, surface to volume to volume ratio (SVR) 17.5]. This is much higher than a glass capillary reaction tube (80 mm^2:10 µl, SVR 8) or a conventional plastic reaction rube (77mm^2 :50µl, SVR 1.54). A high surface to volume ratio is advantageous, allowing for more efficient thermal conduction and dissipation. This high thermal transfer should translate into faster cycling times in the microchips compared with what is currently achievable in the Gene Amp ™ reaction tubes or in glass capillary tubes. The shortest cycling time for PCR achieved by conventional PCR hardware using a positive displacement plastic tip as the reaction vessel is 30 min for a total of 30 cycles (27). The SVR of the reaction tip is 4.7 mm^2/µl (total reaction volume 10 µl) and is smaller than the SVR of the microchip. It is therefore anticipated that by using the PCR microchip, which has a larger SVR, an even shorter thermal cycling time may be obtained. However, a higher SVR increases the significance of surface chemistry, which may reduce the

> efficiency or inhibit PCR in the microchips. A silicon dioxide surface minimizes this effect (23,24)."

It is therefore clear that the microfabricated chips discussed by Cheng et al. are not intended to be used to immobilize PCR reagents. On the contrary, Cheng teaches away from this. For example in the paragraph bridging pages 383 and 384, it is stated that:

> "In summary, we have confirmed that a silicon dioxide surface in a silicon chip is capable of reducing non-specific adsorption of PCR reagents compared with other silicon surfaces."

If there is even a possibility of adsorption of PCR reagents onto the chip then Cheng recommends using an antibody to prevent this. For example, at the end of the paragraph bridging pages 382 and 383 it is stated that:

> "Without the TaqStart antibody the active site of the Taq DNA polymerase may be non-specifically bound to the glass surface of the microchip, thereby completely losing activity."

This is a still further example of Cheng teaching away from performing PCR or other amplification procedures immobilized reagents.

Turning to Hahn et al, this reference also does not provide suggestion or motivation that would lead one to the presently claimed invention. In Hahn et al., PCR is performed in a standard PCR tube rather than on an immobilized surface (see the description of PCR at page 239 and 240). Immobilization occurs only after PCR has been completed. Immobilization is used so that certain PCR products that have not been cleaved in an earlier selective restriction enzyme digestion ("RED") step can readily be quantitated. They carry a digoxigenin group and can be detected by ELISA using a digoxigenin specific Fab - horseradish peroxide conjugate. This is clear from, e.g., Figure 2 of Hahn et al.

Thus, immobilization is used in Hahn et al. for a completely different purpose from the present invention and PCR is performed within a fluid environment rather than on a surface. Hahn therefore also leads away from the present invention.

From the above, it is readily apparent that none of the cited art teaches or suggests the features of the presently claimed methods, e.g., hybridizing extended,

immobilized primers with adjacent primers for further amplification. Rather, the discussions in the cited references expressly teach away from the present invention. Accordingly, withdrawal of the instant rejection is respectfully requested.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400 x 5209.

Respectfully submitted,

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